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Use of Supplemented Stainer-Scholte Broth for the Isolation of *Bordetella pertussis* from Clinical Material

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The use of Stainer-Scholte broth supplemented with (2,6-*O*-dimethyl)β-cyclodextrin (heptakis) for the isolation of *Bordetella pertussis* from clinical specimens was evaluated with 3,632 nasal swabs from children and adults with suspected whooping cough or from their family contacts. The liquid enrichment medium was subcultured on charcoal agar with 10% defibrinated horse blood. Charcoal agar and soft charcoal agar served as the standard procedure to detect *B. pertussis*. We isolated 772 strains of *B. pertussis* (21%). Charcoal agar alone detected 87% of all strains ($n = 668$), soft charcoal agar grew 78% ($n = 602$), and 637 strains (83%) were isolated when Stainer-Scholte broth with heptakis was used. We detected 590 isolates with all three media. Whereas 65 strains grew only on charcoal agar, 27 strains were detected by soft charcoal agar. Supplemented Stainer-Scholte broth allowed the isolation of an additional 77 strains which did not primarily grow on charcoal media ($P < 0.05$). Our data indicate that Stainer-Scholte medium supplemented with heptakis can be effectively used as an enrichment medium for detection of *B. pertussis* in clinical specimens.

Isolation of *Bordetella pertussis* from clinical material can be performed effectively with the original medium of Bordet and Gengou (BG medium) which was described in 1906 (2). Additionally, different charcoal media are frequently used (5, 8, 9, 11, 15). In 1970, Stainer and Scholte (13) described a synthetic liquid medium for the culturing of *B. pertussis* (SS medium), which is now widely used for vaccine preparation. Growth of *B. pertussis* in SS medium, however, could be achieved only when large bacterial inocula were used. Imaizumi and co-workers (6, 7) found that growth and toxin production of *B. pertussis* in SS medium could be significantly increased by heptakis(2,6-*O*-dimethyl)β-cyclodextrin. Heptakis was furthermore shown to be capable of reducing the size of the inoculum needed to facilitate growth of *B. pertussis* (6).

Since we thought that this medium might be useful in isolating *B. pertussis* from clinical specimens, we attempted to evaluate whether a modified SS medium could be used effectively in routine bacteriology of whooping cough in an endemic area such as the Federal Republic of Germany.

MATERIALS AND METHODS

Swabs. Nasal swabs were taken from children and adults with suspected whooping cough or from their family contacts. The swabs were made of calcium alginate which was attached to a thin, flexible drilled wire. Ready-made Amies medium with charcoal served as a transport medium (MAST-Diagnostika, Hamburg, Federal Republic of Germany). Preliminary experiments had shown that Amies medium with charcoal yielded isolation rates similar to those of Regan and Lowe medium (11) when the transport time did not exceed 24 h. Nasal swabbing was demonstrated to the pediatricians individually by members of our institute. The time interval between collection of the swabs and their arrival at the laboratory was usually less than 24 h. Nasal swabs from the right and left nostrils of one patient were received in two separate transport tubes and processed separately but counted as a single specimen. For most patients (95%), however, only a single nasopharyngeal swab was sent.

Routine processing of specimens in the laboratory. Routine processing was designed on the basis of the results of initial experiments (see below). All swabs were first streaked onto a charcoal agar (CH) plate (Oxoid CM 119 with addition of 10% defibrinated horse blood) containing 40 μg of cephalexin per ml (8, 14) and then onto a blood agar plate (Oxoid, Wesel, Federal Republic of Germany) with 10% defibrinated sheep blood. The swabs were then immersed in soft charcoal agar (CB) (Oxoid CM 119; 0.25× with 10% defibrinated horse blood and 40 μg of cephalexin per ml) made as described by Regan and Lowe (11).

Finally, the swabs were immersed in SS broth (13) composed as described below and containing in addition 1 g of heptakis (Tejin Chemicals, Tokyo, Japan; also available from Sigma Chemical Co., St. Louis, Mo.) (6). SS broth was prepared by mixing 1,000 ml of basal medium (10.71 g of glutamic acid, 0.24 g of proline, 2.50 g of NaCl, 0.50 g of KH_2PO_4, 0.20 g of KCl, 0.10 g of $MgCl_2$, 0.02 g of $CaCl_2$, 1.52 g of Tris dissolved in double-distilled water) and 10 ml of supplement (4.00 g of L-cysteine dissolved in 120 ml of 1 N HCl with subsequent addition of double-distilled water to 1,000 ml and 1.00 g of ferrous sulfate (heptahydrate), 2.00 g of ascorbic acid, 0.40 g of niacin, and 10.0 g of reduced glutathione [the solution was sterilized by membrane filtration]). When basal medium and supplement were mixed, the broth was stable for 1 week at +4°C.

Reading of cultures. The blood agar plate was read after 24 and 48 h of incubation at 37°C. Growth of *Haemophilus influenzae*, pneumococci, beta-hemolytic streptococci, or *Staphylococcus aureus* in pure culture was reported. CH plates were incubated at 37°C in ambient atmosphere and read daily for 7 days. CB and SS broths were incubated for 48 h at 37°C in ambient atmosphere and then streaked onto CH plates after vortexing of the SS broth. Subsequently, these CH plates were incubated as described above and read daily for 5 days. Plates were not read on Saturdays and Sundays.

Differentiation of *B. pertussis*. Bacterial growth on CH plates was differentiated by Gram stain, culture morphology,

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TABLE 1. Ability of different media to grow 30 clinical isolates of *B. pertussis*[a]

Medium	No. of strains that grew	% Growth	Time (days)
BG1	30	100	2.8
BG2	21	70	3.6
CH	30	100	3.0
SA1	9	30	4.2
SA2	27	90	3.8
SS1	16	53	4.9
SS2	30	100	3.8
CB	29	97	4.0

[a] Enrichment media (SS1, SS2, and CB) were streaked in CH after 48 h of incubation at 37°C.

TABLE 2. Isolation of *B. pertussis* and *B. parapertussis* from clinical specimens with different media

Organism and medium	No. (%) of isolates that grew:	
	On the indicated medium	Only on the indicated medium[a]
B. pertussis		
CH	668 (87)	65 (8)
CB	602 (78)	27 (4)
SS2	637 (83)	65 (8)
B. parapertussis		
CH	48 (92)	
CB	52 (100)	4 (8)
SS2	48 (92)	

[a] Of the *B. pertussis* isolates, 12 (2%) grew only on CB and SS2.

and direct immunofluorescence tests for *B. pertussis* and *B. parapertussis*. Immunofluorescence was performed with fluorescein isothiocyanate-coupled antisera to *B. pertussis* and *B. parapertussis* (Difco Laboratories, Detroit, Mich.). Stock cultures of both species served as positive and negative controls in every test. Bacteria with a typical shape, typical apple-green fluorescence, and typical Gram staining were further tested for oxidase production. For several isolates, specific bacterial agglutination was used as an additional differentiation criterion (Wellcome, Grossburgwedel, Federal Republic of Germany; antisera to *B. pertussis* and *B. parapertussis*).

In preliminary experiments, different media were compared to choose a solid medium and an enrichment medium for routine culture. Self-made BG medium (BG1), ready-made BG medium (BG2; Oxoid), SS broth without heptakis (SS1), Stainer-Scholte agar (1.5% agar; Oxoid) (SA1), and SA1 with 1 g of heptakis per liter (SA2) were used together with the other media described above.

Stock cultures from 30 strains of *B. pertussis* which had been isolated from clinical material were plated onto CH without antibiotics. One colony of each strain was immersed in 2 ml of SS broth with heptakis and incubated for 48 h at 37°C. One loopful (5 μl) was streaked onto solid medium or immersed in liquid medium. Reading of plates and subculturing of liquid medium were performed as described above.

Statistical evaluation. Statistical evaluation was done by the chi-square test.

RESULTS

Internal evaluation. Thirty clinical isolates of *B. pertussis* were grown on different media to allow preselection before the clinical evaluation. Table 1 shows the results of this initial comparison. CH was equivalent to self-made BG medium but superior to ready-made BG medium and both SA1 and SA2.

A comparison of the three enrichment media showed that CB and SS2 were equivalent but that both were superior to SS1.

As a consequence, on the basis of these data and in accordance with previously published observations (4, 11, 14, 15), clinical specimens were processed with CH, CB, and SS2.

Clinical evaluation. During a period of 24 months, 772 strains of *B. pertussis* and 52 strains of *B. parapertussis* were isolated (Table 2). The mean isolation time of *B. pertussis* was 3.65 ± 1.35 days, and that of *B. parapertussis* was 2.81 ± 1.20 days. Table 2 lists the number of *B. pertussis* and *B. parapertussis* isolates relative to the different media. CH medium alone was able to detect 87% of all *B. pertussis* strains. Use of SS2 increased the isolation rate by 10% (65 + 12 isolates; $P < 0.05$), while CB detected 27 additional strains. CH plates alone grew 92% of all *B. parapertussis* strains (52 isolates). SS2 was ineffective in increasing the isolation rate, while CB facilitated the growth of four additional strains (8%).

A total of 637 strains (83%) of *B. pertussis* and 48 strains of *B. parapertussis* grew in SS2, while 602 strains of *B. pertussis* and 52 strains of *B. parapertussis* grew in CB. Table 3 shows the lengths of time needed for *B. pertussis* and *B. parapertussis* isolation on the different media. Compared with the data of Table 1, no significant differences were found. Since the mean time between collection of the swabs and their arrival in the laboratory was less than 24 h, we were able to use a transport medium without antibiotics. Table 4 summarizes the cultivation of other potentially pathogenic bacteria from the nasal swabs. As expected, pneumococci, *Haemophilus influenzae*, *Staphylococcus aureus* and beta-hemolytic streptococci made up more than 95% of all possibly pathogenic isolates apart from *B. pertussis*. The remainder consisted of monocultures of *Pseudomonas* spp., *Enterobacteriaceae*, and *Candida* spp.

DISCUSSION

As long as 82 years after the original Bordet and Gengou paper (2), the medium which they described is still one of the most effective means of isolating *B. pertussis* from clinical material. Growth of *B. pertussis* is inhibited by unsaturated

TABLE 3. Appearance of macroscopically visible colonies of *B. pertussis* or *B. parapertussis* with different culture and enrichment methods

Organism and medium	Mean ± SD time of isolation (days)
B. pertussis	
CH	3.2 ± 0.9
CB	4.0 ± 0.9
SS2	3.7 ± 1.2
B. parapertussis	
CH	2.5 ± 1.1
CB	3.2 ± 1.0
SS2	3.5 ± 1.3

TABLE 4. Cocultivation of other bacteria from nasal swabs sent for isolation of *B. pertussis*

Microorganism(s)	No. (%) of swabs	
	Without *B. pertussis* or *B. parapertussis*[a]	With *B. pertussis* or *B. parapertussis*[a]
Streptococcus pneumoniae	650 (23)	148 (18)
Staphylococcus aureus	340 (12)	90 (11)
Haemophilus influenzae	288 (10)	62 (8)
Beta-hemolytic streptococci	49 (2)	8 (1)
Other possibly pathogenic microorganisms	51 (2)	17 (2)

[a] There were 2,808 swabs without and 824 swabs with *B. pertussis* or *B. parapertussis*.

fatty acids, colloidal sulfur or sulfides, and organic peroxides (cf. reference 12). These inhibitors can be absorbed by starch, albumin, or charcoal. Consequently, different culture media for *B. pertussis* have been proposed, although only charcoal medium has found wide acceptance. Stainer and Scholte formulated a synthetic medium without absorbent which was primarily intended for mass culture of large inocula and is used mainly for vaccine production (13). Imaizumi and co-workers reported that growth of *B. pertussis* and production of exotoxins in SS medium can be significantly increased when heptakis is added (6, 7).

In accordance with the data of Stainer and Scholte (13) and Imaizumi et al. (6), we found that large inocula were needed for unsupplemented SS broth but that addition of heptakis facilitated isolation from small inocula significantly. Preliminary experiments showed furthermore that SS broth with heptakis subcultured on CH yielded better results than SS agar with heptakis. These data (Table 1) are also in accordance with findings of Aoyama and co-workers (1), who showed that SS agar with heptakis is superior to ready-made BG medium.

Consequently, we evaluated SS broth with heptakis for its ability to grow *B. pertussis* and *B. parapertussis* from clinical specimens. Sensitive and simple methods to detect *B. pertussis* are especially needed in nonvaccinating countries such as the Federal Republic of Germany.

We evaluated SS broth with more than 3,600 clinical specimens and found that SS broth supplemented with heptakis facilitated the isolation of 65 strains (6%) which were not detected by either CH or CB.

Our data indicate that, in addition to the routine procedure of isolating *B. pertussis* (3, 10), SS broth supplemented with heptakis can effectively increase the isolation rate of *B. pertussis* from clinical specimens. Finally, our data suggest that the use of an antibiotic-free transport medium may allow frequent isolation of other potentially pathogenic microorganisms from nasopharyngeal swabs. However, this seems feasible only when a short transport time can be guaranteed. Furthermore, these findings need to be validated in additional comparative studies.

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LITERATURE CITED

1. **Aoyama, T., Y. Murase, T. Iwata, A. Imaizumi, Y. Suzuki, and Y. Sato.** 1986. Comparison of blood-free medium (cyclodextrin solid medium) with Bordet-Gengou medium for clinical isolation of *Bordetella pertussis*. J. Clin. Microbiol. **23**:1046–1048.
2. **Bordet, J., and U. Gengou.** 1906. Le microbe de la coqueluche. Ann. Inst. Pasteur. **20**:731–741.
3. **Finger, H.** 1986. *Bordetella*, p. 386–390. *In* S. Baron (ed.), Medical microbiology. Addison-Wesley, Menlo Park, Calif.
4. **Gilligan, P. H., and M. C. Fisher.** 1984. Importance of culture in laboratory diagnosis of *Bordetella pertussis* infections. J. Clin. Microbiol. **20**:891–893.
5. **Holwerda, J.** 1971. Symposium on pertussis immunization, in honor of Dr. Pearl L. Kendrick in her eightieth year: current diagnostic procedures in whooping cough. Health Lab. Sci. **8**: 206–209.
6. **Imaizumi, A., Y. Suzuki, S. Ono, H. Sato, and Y. Sato.** 1983. Heptakis(2,6-*O*-dimethyl)β-cyclodextrin: a novel growth stimulant for *Bordetella pertussis* phase I. J. Clin. Microbiol. **17**:781–786.
7. **Imaizumi, A., Y. Suzuki, S. Ono, H. Sato, and Y. Sato.** 1983. Effect of heptakis(2,6-*O*-dimethyl)β-cyclodextrin on the production of pertussis toxin by *Bordetella pertussis*. Infect. Immun. **41**:1138–1143.
8. **Lautrop, H., and B. W. Lacey.** 1960. Laboratory diagnosis of whooping cough or *Bordetella* infections. Bull. W.H.O. **23**:15–35.
9. **Mishulov, L., L. S. Sharpe, and L. L. Cohen.** 1953. Beef-heart charcoal agar for the preparation of pertussis vaccines. Am. J. Public Health **43**:1466–1472.
10. **Parker, C. D., and B. J. Payne.** 1985. *Bordetella*, p. 394–399. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
11. **Regan, J., and F. Lowe.** 1977. Enrichment medium for the isolation of *Bordetella*. J. Clin. Microbiol. **6**:303–309.
12. **Rowatt, E.** 1957. The growth of *Bordetella pertussis*: a review. J. Gen. Microbiol. **17**:297–326.
13. **Stainer, D. W., and M. J. Scholte.** 1970. A simple chemically defined medium for the production of phase I *Bordetella pertussis*. J. Gen. Microbiol. **63**:211–220.
14. **Stauffer, L. R., D. R. Brown, and R. E. Sandstrom.** 1983. Cephalexin-supplemented Jones-Kendrick charcoal agar for selective isolation of *Bordetella pertussis*: comparison with previously described media. J. Clin. Microbiol. **17**:60–62.
15. **Sutcliffe, E. M., and J. D. Abbott.** 1972. Selective medium for the isolation of *Bordetella pertussis* and *parapertussis*. J. Clin. Pathol. **25**:732–733.